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Acmella oleracea extract increases collagen content and organization in partially transected tendons

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Abstract

Acmella oleracea contains spilanthol as the main active compound, which possesses analgesic and anti-inflammatory effects that can favor tendon reorganization. To analyze the effect of *A. oleracea* on the content and organization of collagen in injured tendons, the calcaneal tendon of male Lewis rats was partially transected and treated at the site of injury with a topical application of 20% *A. oleracea* ointment (AO group) or with the ointment base without the plant extract (B group). The animals were euthanized 21 days after partial transection. Higher collagen concentration was observed in the AO group than in the B group, and morphological analysis using polarization microscopy showed higher birefringence in the AO group than in the B group, indicating higher collagen organization. No difference was observed in the number of fibroblasts, blood vessels, proteoglycan distribution, and maximum load between the B and AO groups. In conclusion, topical application of 20% *A. oleracea* ointment increased the molecular organization and content of collagen, thus indicating a potential application in tendon repair. Studies on the later phases of the tendon healing process are necessary to demonstrate the possible biomechanical changes after the application of *A. oleracea* ointment.

KEYWORDS
Acmella oleracea, birefringence, calcaneal tendon, repair

1 | INTRODUCTION

The *Acmella oleracea* (L.) R. K. Jansen, synonym *Spilanthes acmella* (L.) L., popularly known as jambu, belongs to the *Asteraceae* family and is native to the Amazon region. *A. olleracea* is very useful in folk medicine for the treatment of diseases in the mouth, throat, and teeth, rheumatism, anemia, and influenza, among others, because of bioactive compounds such as spilanthol (de Freitas-Blanco et al., 2019; Neves et al., 2019). The chemesthetic sensation in the oral mucosa after consumption of the plant is due to the presence of alkylamides, represented by a high concentration of spilanthol, which is responsible for its analgesic and anti-inflammatory effects (Dallazen et al., 2018; de Freitas-Blanco et al., 2019). Certain in vivo and in vitro studies have described the anti-inflammatory and antinociceptive effects of the extract obtained from *A. oleracea* or isolated spilanthol, and enhanced wound contraction in the skin of rats (Dallazen et al., 2020; Huang, Huang, Hu, Peng, & Wu, 2019; Cho, Bach, Kim, Vuong, & Cho, 2017; Yamane et al., 2016; Nomura et al., 2013).

The active compounds present in plant extracts have great pharmacological potential for the treatment of tendon injuries, which can act in the compositional, structural, and functional recovery of this tissue (Maria-Ferreira et al., 2014; Maria-Ferreira et al., 2018; Yamane

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et al., 2016). Following an injury, the tendon undergoes a repair process that involves numerous molecular and cellular events, which can be divided into the inflammatory, proliferative, and remodeling phases (Docheva, Müller, Majewski, & Evans, 2015; Murphy, Loitz, Frank, & Hart, 1994; Nourissat, Berenbaum, & Duprez, 2015; Oakes, 2003; Sharma & Maffulli, 2006). The remodeling phase starts approximately on the 21st day after tendon injury in animal models (Sharma & Maffulli, 2005). The abundant extracellular matrix (ECM) of tendons is mainly composed of collagen fibers, glycosaminoglycans, proteoglycans, and glycoproteins, among other molecules, and forms a highly organized supramolecular structure that is capable of supporting the biomechanical demands of the tissue (Sharma & Maffulli, 2005; Vidal & Mello, 1984; Vidal & Mello, 2010). Collagen is hierarchically organized, starting with the tropocollagen molecule that forms fibrils, fibers, bundles, and fascicles (Guerquin et al., 2013; Sharma & Maffulli, 2006). The remodeling phase predominantly involves the restoration of collagen content and organization (Docheva et al., 2015). Depending on the extent of the lesion, the healed tendon may or may not present a complete recovery of the biochemical and ultrastructural characteristics and properties even after 1 year of the lesion (Yang, Rothrauff, & Tuan, 2013). The formation of a fibrous scar at the site of injury may favor a high incidence of re-rupture (Farkas et al., 1973; Hooley & Cohen, 1979).

Studies performed by our group have shown beneficial effects of topical application of certain plant extracts in the recovery of transected calcaneal tendon, up to the 21st day of the healing process. Among them, the application of *A. vera* ointment increased the amount of metalloproteinase-2, non-collagen protein concentration, and content and organization of glycosaminoglycans and collagen fibers (Aro et al., 2012; Aro et al., 2014). The *A. chica* extract stimulated the synthesis and activation of metalloproteinase 2, increased the organization of collagen, and the amount of dermatan sulfate, thereby improving tissue remodeling and gait of animals (Aro et al., 2013; Aro et al., 2013). In another study, *C. officinalis* cream increased the concentration of non-collagen proteins as well as the concentration and organization of collagen (Aro et al., 2015). Several studies have also demonstrated the beneficial effects of other plant extracts on the recovery of injured tendons. The application of flavone injections of *H. rhamnoides* on the rat knee increased deposition and maturation of collagen and restored the biomechanics of the injured patellar tendon of rats 14 days after injury (Fu et al., 2005). Aiyegbusi, Duru, Anunobi, Noronha, and Okanlawon (2011)) and Aiyegbusi, Olabiyi, Duru, Noronha, and Okanlawon (2011) administered bromelain, an enzyme extracted from the pineapple stem, by gavage to rats and revealed stimulated fibroblast proliferation on the 15th day after acute tendon injury caused by crushing (Aiyegbusi, Duru, et al., 2011; Aiyegbusi, Olabiyi, et al., 2011). According to Bingbing et al. (2011), a 16% solution of *S. yellow* reduced edema in the transected tendons of chicken until the sixth week, and reduced hyperemia, the inflammatory reaction, and adhesion formation, while increasing biomechanics (Liu et al., 2011).

In addition to the use of plant extracts, other treatments have shown promising effects for the treatment of tendon injuries, such as the use of biomaterials, acupuncture, cell therapy, low-level laser, and ultrasonic therapy. These studies have demonstrated beneficial effects on tendon recovery in different injury models, such as greater organization of collagen fibers, greater expression of decorin, greater activity of metalloproteinases, increased cell density, reduced tendon adhesion, and increased tendon resistance (Lucke et al., 2019; Frauz et al., 2019; Liu et al., 2019; Xie et al., 2019; Liu, Yu, Bai, Tian, & Liu, 2018; de Aro et al., 2018; Farcic et al., 2018; Chamberlain, Saether, Aktas, & Vanderby, 2017; Yang et al., 2017; Almeida, Oliveira, Vieira, Guerra, & Pimentel, 2016; Teng, Zhou, Xu, & Bi, 2016; Guerra et al., 2013; de Almeida et al., 2012; Chen et al., 2009; Ng & Fung, 2007; Ng, Ng, See, & Leung, 2003). Despite the promising treatments demonstrated in several studies, the intrinsic hypocellularity and hypovascularization of tendons limit their natural healing process, which is slow, complex, and often ineffective, and may lead to functional loss (Gonçalves et al., 2013).

Thus, it is necessary to search for new treatments aiming to improve tendon repair. We hypothesized that *A. oleracea* ointment, due to its anti-inflammatory effects, could accelerate tendon repair by enhancing tissue restructuring. There are no studies demonstrating the effects of *A. oleracea* on the repair of highly fibrous and organized tissues, such as tendons. Thus, the present study aimed to analyze the effects of topical application of *A. oleracea* ointment (20%) on the repair process of the calcaneal tendon in rats, a phase characterized by tissue remodeling, which involves increase in the content and organization of total collagen. Collagen represents approximately 80% of the tendon constitution, and therefore treatments that accelerate collagen restoration and organization could be considered promising tools for tissue repair.

2 | MATERIALS AND METHODS

2.1 | Crude *A. oleracea* extract

The ointment produced with the crude *A. oleracea* extract used in the present study was provided by CPQBA-UNICAMP (Pluridisciplinary Center for Biological and Agricultural Research, State University of Campinas), located in the city of Paulínia-SP (22° 45 00″ South and 47° 10 21″ West).

All leaves and flowers of *A. oleracea* were collected at the same time, dried at 45°C in an oven with forced air circulation, and ground in a hammer mill, followed by extraction with 96° GL ethanol (1:5, w/v) in a stainless steel tank with mechanical stirring for 4.5 hr (three consecutive times for 1.5 hr each with solvent renewal). The solid residue was separated from the extract through filtration of each of the extractions (Rodrigues et al., 2006). The partial extracts obtained were filtered, combined, and concentrated under vacuum in a rotary evaporation system at a temperature of 40°C. The extract was then lyophilized to a constant mass for calculating extraction yield, packed in an amber bottle, and stored in a freezer for future analysis. The *A. oleracea* lyophilizate was added to a base ointment (of anhydrous lanolin and solid vaseline 30:70 w/w) (Aro et al., 2012), at a concentration of 20% w/w.

2.2 | Experimental groups

In the present study, 39 male Lewis rats (90 days old) were used and fed with food and water ad libitum. The animals were separated into three experimental groups ($n = 13$ in each group): normal (N) group, composed of rats with calcaneal tendons without transection euthanized at the same age as the transected groups (111 days); base (B) group, composed of rats with tendons transected and treated with 64 mg of the topical applications of the ointment base without the extract, starting on the day of the transection and on three consecutive days after transection, totalizing four applications, and euthanized on the 21st day after the injury; 20% *A. oleracea* ointment (AO) group, composed of rats with transected tendons and treated with 64 mg of the topical applications of *A. oleracea* ointment (20%) on the day of the transection and on three consecutive days after transection, totalizing four applications, and euthanized on the 21st day after injury (Aro et al., 2015). This study was performed according to the Institutional Committee for Ethics in Animal Research of the State University of Campinas—UNICAMP (protocol no. 5511-1/2020).

2.3 | Protocols for partial transection of the calcaneus tendon and topical application of 20% *A. oleracea* ointment

The animals were anesthetized with intraperitoneal injection of ketamine hydrochloride (80 mg/kg) and xylazine hydrochloride (10 mg/kg), and the lower right paws were subjected to trichotomy and antisepsis with iodized alcohol. A longitudinal incision was made in the skin to expose the calcaneal tendon and a partial transection was performed in the proximal region of the tendon, located at a distance of approximately 4 mm from the incision of the calcaneus tendon (Aro et al., 2012; Aro et al., 2014; Aro et al., 2015; Aro, Freitas, et al., 2013; Aro, Simões, et al., 2013; Lucke et al., 2019). To completely cover the tendon transection region, 64 mg of *A. oleracea* ointment (20%) was applied at the site of injury before the skin was sutured. After ointment application, the skin was sutured with a nylon suture thread (Shalon 5-0) and needle (1.5 cm). From the first day after tendon transection, topical application of 64 mg of the *A. oleracea* ointment (20%) was performed daily on the sutured skin and massaged gently for its absorption. This procedure was repeated until the third day after surgery, with a total of four topical applications. The same procedure was performed for the transected tendon controls that received 64 mg of ointment base, without the extract of *A. oleracea*. A rat retainer (Request Privilege of Utility Model in Brazil, titled: Retainer of rats for laser beam and biocompounds application, deposited in the National Institute of Industrial Property INPI, on 04.16.10, under no. MU9000622-4, authored by researchers Andrea Aparecida de Aro and Edson Rosa Pimentel, from the Institute of Biology, UNICAMP) was used for the immobilization of the animals during daily topical applications.

2.4 | Preparation of sections for freezing and hematoxylin–eosin and toluidine blue staining

Tendons were collected, embedded in Tissue-Tek®, and frozen. Longitudinal sections of 12 μm were obtained using a cryostat. The sections were fixed using 4% formaldehyde solution in Millonig buffer (0.13 mol/L sodium phosphate and 0.1 mol/L sodium hydroxide, 7.4 pH) for 20 min, followed by HE and 0.025% TB staining in McIlvaine buffer (0.03 M citric acid, 0.04 M sodium phosphate dibasic, pH 4.0) (Mello & Vidal, 2003). Images of the tendons were obtained using an Olympus BX53 microscope and analyzed using an image analyzer (Life Science Imaging Software, Olympus Soft Imaging Solutions GmbH, Münster, Germany Version 510_UMA_cellSens16_Han_en_00).

2.5 | Morphometry

Images from longitudinal sections of the transection region of the tendons were captured and digitized using a Leica DM 2000 photomicroscope. Digitized images were used to determine the total number of fibroblasts and blood vessels ($n/10^4\ \mu m^2$). Five samples were collected from the central region of the transection in each animal ($n = 3$) using a virtual Leica Image Measure TM grid.

2.6 | Polarization microscopy: Birefringence measurements

After fixation, the organization of the collagen bundles of tendons ($n = 4$) from each group were analyzed based on the variation in birefringence intensity. Since birefringence appears visually as brilliance, this phenomenon was measured with an image analyzer and expressed as gray average (GA) values in pixels (8 bits = 1 pixel). The collagen bundles exhibit two types of birefringence: intrinsic birefringence (Bi) and form or textural birefringence (Bf) (de Campos Vidal, 1980; Vidal, 1980; Vidal, 1986). Total birefringence (the sum of Bi and Bf) was used in this study, for which the larger tendon axis was positioned at 45° to the crossed analyzer and polarizer. Measurements of GA (an average of 390) of the transected region (TR) of tendons from each experimental group were performed after immersing the sections in water, a condition in which total birefringence is highly detectable (Vidal & Mello, 2010; Vidal, 1986; de Campos Vidal, 1980; Vidal, 1980). The birefringence properties were studied using an Olympus BX53 polarizing microscope and an image analyzer (Life Science Imaging Software, Version 510_UMA_cellSens16_ Han_en_00).

2.7 | Biomechanical parameters

Tendons from experimental groups ($n = 9$) were collected and stored at −20°C until tested. Before the biomechanical test, the tendons were thawed and measured with a pachymeter, considering their length, width,

and thickness. For the biomechanical test, the tendons were maintained in PBS to prevent drying of their fibers. They were then fixed to the metal claws by the myotendinous and osteotendinous junction for correct alignment in the equipment. A texturometer (MTS, model TESTSTAR II) was used for this analysis. In each test, tendons were subjected to a gradual increase in load at a displacement velocity of 1 mm/s using a load of 0.05 N until the tendon ruptured, according to the protocols of Sparrow, Finucane, Owen, and Wayne (2005) and Benevides et al. (2004). The test provided maximum load results (N).

2.8 | Hydroxyproline quantification

Whole tendons from the biomechanical test ($n = 9$) were cut and immersed in acetone for 48 hr, followed by chloroform: ethanol (2:1) for 48 hr. The fragments were weighed and hydrolyzed in 6 M HCl (1 m/10 mg of tissue) for 4 hr at 130°C. Then, 6 M NaOH was added to neutralize the hydrolysate, and hydroxyproline quantification was performed as described by Stegemann and Stalder (1967) (Stegemann & Stalder, 1967) and Jorge et al. (2008) (Jorge et al., 2008), with some modifications. The amount of hydroxyproline used for the standard curve varied between 0.2 and 6 μg and the absorbance of the different samples was measured at 550 nm using a microplate reader (Expert Plus; Asys, Holliston, MA).

2.9 | Statistical analysis

Data with normal distribution (Gaussian distribution) were analyzed using the analysis of variance (one-way ANOVA) followed by the Tukey test ($p < .05$): hydroxyproline quantification and biomechanical test. For data that did not adhere to the Gaussian distribution, the nonparametric Kruskal–Wallis test was used, followed by the Dunns post-test ($p < .05$): birefringence measurements. All results are represented as the mean ± *SD* and median (in the case of birefringence measurements). GraphPad Prism® (Graph-Pad Software, La Jolla, CA), version 3.0, was used for analysis.

3 | RESULTS

3.1 | Collagen fiber reorganization

Higher birefringence values were observed in the transected region (TR) of tendons in the AO group (mean: 65.5 ± 30.9; median: 57.7) compared with that in the B group (mean: 34.9 ± 16.3; median: 31.7). The N group (mean: 217.5 ± 30.0; median: 224.5) presented higher birefringence than the B and AO groups (Figure 1).

3.2 | ECM organization and proteoglycan distribution

Tendon sections from the B and AO groups stained with HE presented higher cellularity compared with that in the N group, mainly in the TR. The B group showed slightly stained regions compared with that in the AO group (Figure 1).

Intense metachromasia was observed in the TR of tendons in the B and AO groups stained with TB, with no marked differences between them. The N group presented slightly stained ECM due to the low proteoglycan content (Figure 2).

3.3 | Fibroblast and blood vessel quantification

Morphometry analysis showed no differences in the number of fibroblasts (B: 125.2 ± 8.2; AO: 124.2 ± 7.8) and blood vessels (B: 1.9 ± 0.9; AO: 2.5 ± 0.8) between the B and AO groups (Table 1).

3.4 | Total collagen concentration

The AO group presented higher hydroxyproline concentration (146.2 mg/g of tissue ±16.6), which infers the total collagen concentration, compared with that in the B group (129.6 ± 16.3). The B and AO groups presented no differences in hydroxyproline concentration compared with the N group (136.7 ± 23.8) (Figure 3).

3.5 | Biomechanical resistance of tendon: Maximum load (N)

No significant differences were observed in the maximum load (N) values supported by tendons between the N (17.4 ± 4.3), B (20.2 ± 8.2), and AO (18.5 ± 4.5) groups (Figure 4).

4 | DISCUSSION

The literature describes the use of *A. oleracea* as an insecticide (Benelli, Pavel, Drenaggi, & Maggi, 2019), in ectoparasite control (Anholeto et al., 2017; Anholeto et al., 2018; de Oliveira et al., 2019; Marchesini et al., 2018), in the treatment of intestinal mucositis (Maria-Ferreira et al., 2018), and many different effects, including antipyretic (Chakraborty, Devi, Sanjebam, Khumbong, & Thokchom, 2010), anti-inflammatory (Chakraborty, Devi, Sanjebam, Khumbong, & Thokchom, 2004; Wu et al., 2008), analgesic (Ratnasooriya & Pieris, 2005), local anesthetic (Chakraborty et al., 2010), antimicrobial (Arora, Vijay, & Kumar, 2011), antifungal (Rani & Murty, 2006), antimalarial (Bae, Ehrmann, Ettefagh, & Cech, 2010; Mbeunkui et al., 2011), antioxidant (Prachayasittikul et al., 2009; Wu et al., 2008), relaxing vase (Wongsawatkul et al., 2008), diuretic (Gerbino et al., 2016; Ratnasooriya, Pieris, Samaratunga, & Jayakody, 2004), and immunostimulant (Savadi, Yadav, & Yadav, 2010; Wu et al., 2008). However, there are no data in the literature demonstrating the effects of *A. oleracea* extract on the content and organization of collagen, particularly in highly fibrous and

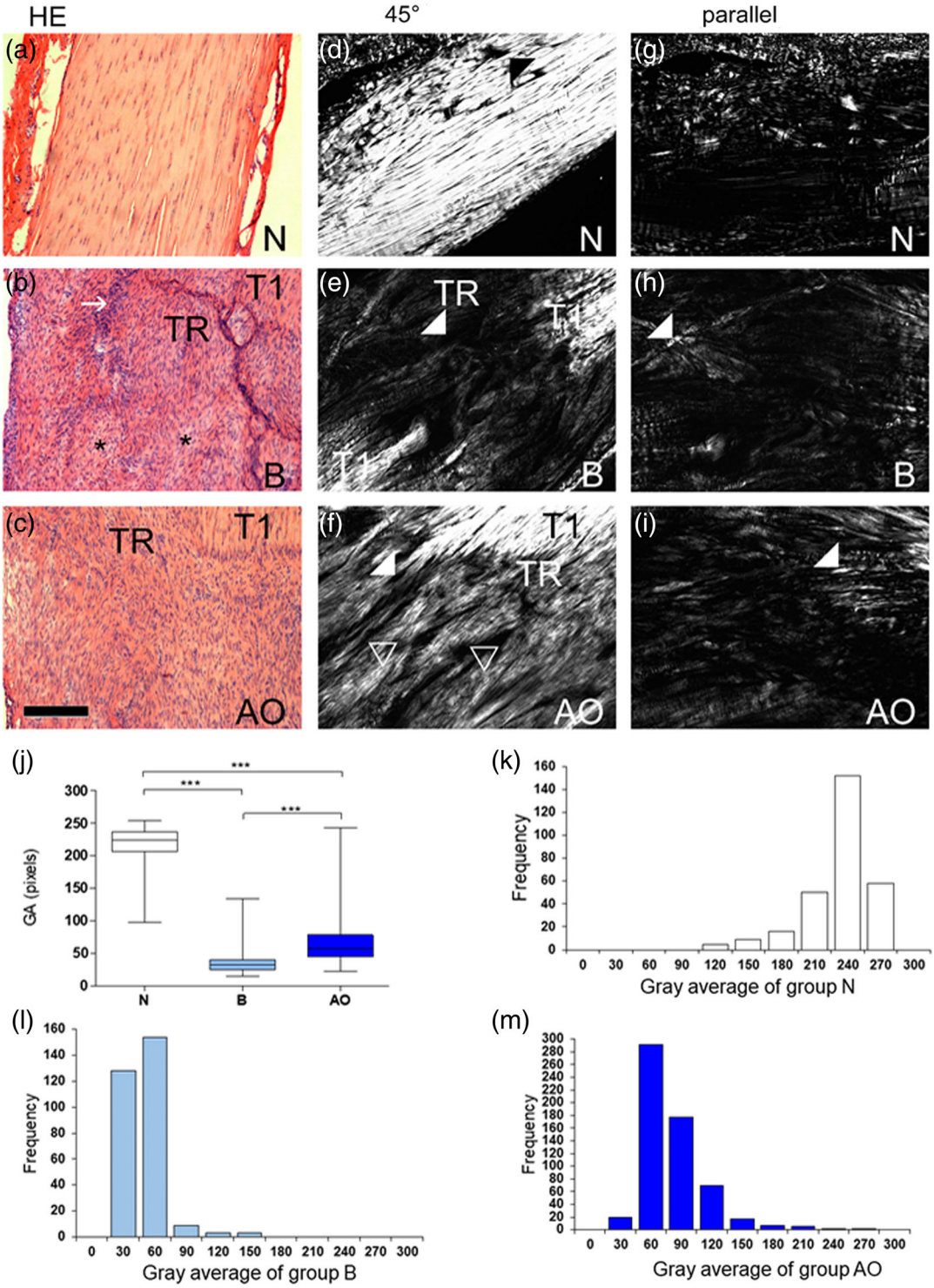


FIGURE 1 Longitudinal sections of the tendons stained with and without hematoxylin–eosin and analyzed using light microscopy (a–c) and polarized light microscopy (d–i), respectively. (a–c) High cellularity is observed in the transected region (TR) of the Base (B) and *A. oleracea* ointment (AO) groups compared with that in the Normal (N) group, and slightly stained regions (*) are observed in the ECM of the B group compared with that of the AO group, indicating lower collagen fiber aggregation. The B group shows regions with higher number of cells (→). (d–f) When the largest tendon axis is positioned at 45° to the crossed polarizers, higher birefringence brightness is observed in the N group compared with that in the B and AO groups. ▷ indicates fibers with higher birefringence. T1: region of overlap between the non-transected portion of the tendon and the TR. (g–i) The largest tendon axis is positioned parallel to one of the crossed polarizers. The regions with differences in the fiber organization are represented by higher birefringence brightness compared with the same tendon region (▶) observed in d and f. Bars: 300 μm. (j) Median values of gray average (GA). Higher GA value was observed in the AO group compared with that in the B group (***$p < .0001$), and higher GA value was observed in the N group compared with that in the B and AO groups (***$p < .0001$). (k–m) Histograms demonstrating the frequency of distribution of GA values in N (k), B (l), and AO (m) groups [Color figure can be viewed at wileyonlinelibrary.com]

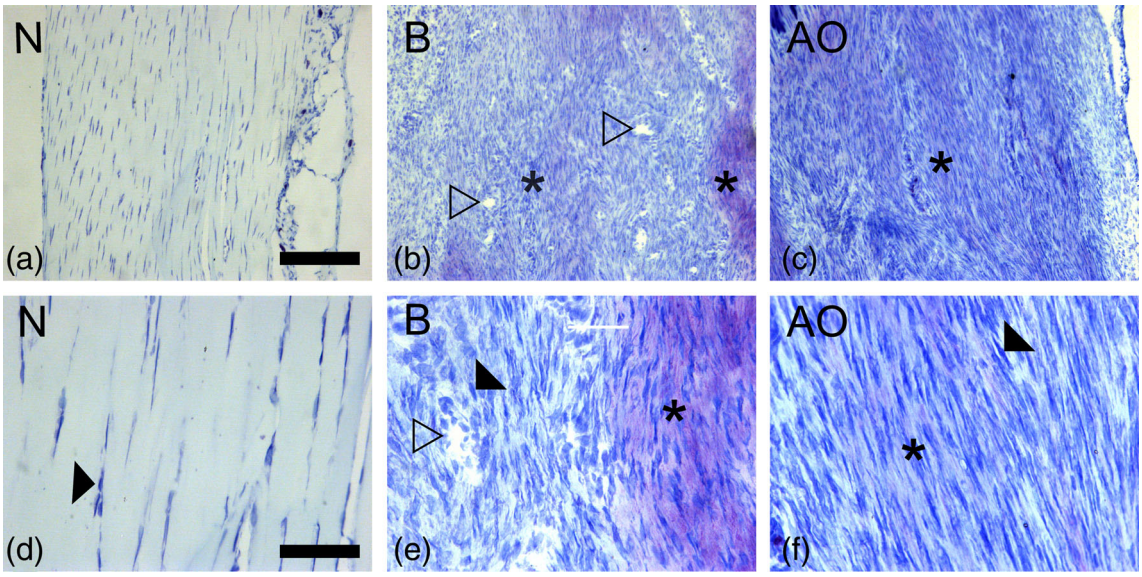


FIGURE 2 Longitudinal sections of tendons stained with toluidine blue and analyzed using light microscopy. Slightly stained ECM is observed in the Normal (N) group (a, d) due to low amount of proteoglycans, with presence of lined fibroblasts (▶). Regions with intense metachromasia (*) are observed in the Base (B) (b, e) and *A. oleracea* ointment (AO) (c, f) groups, without marked differences between them. Higher number of fibroblasts (▶) are observed in the group B and AO compared with that in N group. (▷) indicates blood vessels. (d, e, f) Details of a, b, and c, respectively. Bars: 300 μm (a–c) and 75 μm (d–f) [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Morphometry of the tendons are represented as the mean ($n/10^4 mm^2$) ± *SD*

Groups	*N*	*B*	AO
Fibroblasts	21.3 ± 5.0	125.2 ± 8.2***	124.2 ± 7.8***
Blood vessels	2.1 ± 1.0	1.9 ± 0.9	2.5 ± 0.8

Notes: No significant difference ($p > .05$) was observed in the number of fibroblasts and blood vessels between the Base (B) and *A. oleracea* ointment (AO) groups. Higher number of fibroblasts (***$p < .05$) was observed in the B and AO groups compared with that in the N group.

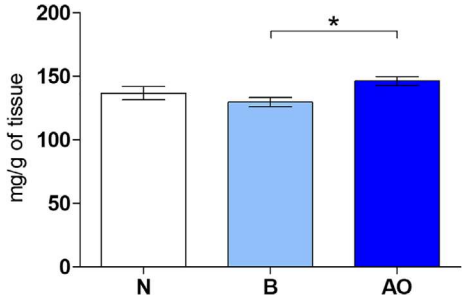


FIGURE 3 Hydroxyproline concentration (mg/g of tissue). Higher concentration of hydroxyproline is observed in the *A. oleracea* ointment (AO) group compared with that in the Base (B) group (*$p < .05$). No significant difference ($p > .05$) is observed in both B and AO groups compared with that in the Normal (N). Values are represented as the mean ± *SD* [Color figure can be viewed at wileyonlinelibrary.com]

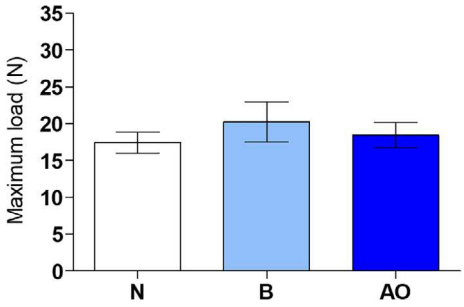


FIGURE 4 Maximum load (N). No significant difference ($p > .05$) is observed between the Normal (N), Base (B), and *A. oleracea* ointment (AO) groups. Values are represented as the mean ± *SD* [Color figure can be viewed at wileyonlinelibrary.com]

organized tissues such as tendons. We hypothesized that, due to the anti-inflammatory effect of the plant extract, a 20% *A. oleracea* ointment could accelerate tendon repair by restoring the content and organization of the collagen matrix, leading to greater biomechanical resistance.

Our results demonstrated a higher concentration of total collagen after application of the *A. oleracea* ointment compared with that in the B group. A study by Yamane et al. (2016) corroborates this result, in which a bioadhesive film containing *A. oleracea* extract associated with the essential oil of *Achyrocline satureioides* was used as a topical application on skin lesions, inducing greater wound contraction and collagen deposition compared with that in the control (Yamane et al., 2016). Furthermore, Maria-Ferreira et al. (2018) demonstrated

that rhamnogalacturonan, a complex polysaccharide extracted from *A. oleracea*, maintained collagen homeostasis in mice colitis (Maria-Ferreira et al., 2018). In another study, Maria-Ferreira et al. (2014) reported that the oral administration of the same polysaccharide had a gastroprotective effect on ethanol-induced rat lesions, in addition to healing effects associated with increased cell proliferation, reduced inflammation, and modulated antioxidant mechanisms (Maria-Ferreira et al., 2014). Our study found no differences in the number of cells and blood vessels upon treatment with the plant extract ointment compared with that in the B group.

The *A. oleracea* ointment-treated group showed greater organization of collagen fibers than the B group. This greater molecular organization of collagen was not reflected in the higher biomechanical resistance of the tendons in the AO group. Although speculative, it is important to emphasize that later stages of the repair process could demonstrate greater biomechanical resistance of the tendons, resulting from the greater structural organization, as demonstrated in our results from the 21st day after the transection. The effects of *A. oleracea* on collagen organization have not been reported in the literature. A possible explanation for the greater structural organization without greater biomechanical resistance is the predominance of collagen fibrils with similar diameters in both groups. Data from Biancalana, Veloso, and Gomes (2010) demonstrated the relationship between different collagen fibril diameters in tendons and variations in tendon strength and elasticity. A study by Frauz et al. (2019) used the same tendon transection model as the one used in the present study, but they treated the tendon with a biodegradable scaffold. The study demonstrated greater organization of collagen fibers on the 21st day after the injury, although no difference was observed in the biomechanical resistance of tendons (Frauz et al., 2019).

The literature describes the anti-inflammatory effects of both *A. oleracea* and spilanthol, its main compound, although its effects on collagen reorganization are unknown. Therefore, considering that there was no significant difference in the distribution of proteoglycans, which act directly on collagen fibrillogenesis (Moorehead, Prudnikova, & Marcolongo, 2019), the greater organization of collagen fibers after ointment application, as observed in our results, may not be related to those components, but to the anti-inflammatory activity of *A. oleracea*, which may have accelerated the healing process. Considering the anti-inflammatory activity of *A. oleracea* in vivo, Dallazen et al. (2020) used a hexane fraction rich in alkylamides extracted from *A. oleracea* flowers for intraplantar application in mice, and observed a potent anti-inflammatory activity, with edema reduction in the mouse's paws following carrageenan-induced inflammation (Dallazen et al., 2020). This study demonstrated that the anti-inflammatory activity involved inhibition of neutrophil migration and pro-inflammatory cytokine release, reduction of oxidative stress and prostaglandin E2 synthesis, and maintenance of IL-10 levels.

With regard to the anti-inflammatory effect of *A. oleracea*, mainly due to spilanthol, Huang, Chang, Hu, Hsiao, and Wu (2018) investigated the in vitro effects of spilanthol on the expression of iCAM-1 induced by TNF-α and COX-2 in keratinocytes, and demonstrated that spilanthol can suppress TNF-α and COX-2. Huang et al. (2018) evaluated the effects of spilanthol on the expression of iCAM-1 and mediators associated with inflammation in the epithelial cells of human lungs, and their results suggested that spilanthol exerts anti-inflammatory effects by inhibiting the pro-inflammatory cytokines COX-2 and iCAM-1, thereby leading to the inhibition of NF-κB and MAPK signaling pathways. A study conducted by de Freitas-Blanco et al. (2019) demonstrated the anti-inflammatory effect of spilanthol on induced intestinal mucositis in mice.

Few studies have described the use of crude *A. oleracea* extracts in vivo. Santana de Freitas-Blanco et al. (2016) confirmed the anesthetic effects of a mucoadhesive film containing 10% or 20% crude *A. oleracea* extracts after application on the oral mucosa of mice (Santana de Freitas-Blanco et al., 2016). Rondanelli et al. (2020) demonstrated that a formulation containing crude *A. oleracea* and *Zingiber officinale* extracts, used as a food supplement for the treatment of knee osteoarthritis, reduced pain due to its anti-inflammatory activity. However, there are no studies using 20% crude *A. oleracea* extracts in tissue repair models (Rondanelli et al., 2020).

In conclusion, although we did not observe biomechanical alteration, our results corroborate our hypothesis since the topical application of 20% *A. oleracea* ointment increased the molecular organization and content of collagen, thus presenting a potential application in tendon repair. Studies on the later stages of the tendon healing process are necessary to demonstrate possible biomechanical changes after the application of *A. oleracea* ointment.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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